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PASADENA, CALIFORNIA

PROGRESS REPORT

on

Neural Control of Hibernation in Mammals; the Relevance
of Physiological Clocks and Environmental Factors.

(NASA Research Grant NGR-05-002-031)

Report #2

Period covered: March 1 - August 31, 1965

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| FACILITY FORM 602 | (ACCESSION NUMBER) | (THRU) |
| | 27 | none |
| | (PAGES) | (CODE) |
| | CR-67339 | (CATEGORY) |
| | (NASA CR OR TMX OR AD NUMBER) | |

This progress report covering the period March 1 - August 31, 1965
includes three papers:

- I. The internal rhythms of hibernations by Felix Strumwasser, Floyd R. Schlechte and John A. Streeter. This is a review paper, written by invitation and to be presented at the IIIrd International Symposium on Mammalian Hibernation (Toronto, Sept. 13-15, 1965).
- II. A Fortran IV computer program to calculate power spectra by Floyd R. Schlechte.
- III. A program to compress, reformat, and summarize the magnetic tape record of several intermixed time series by John A. Streeter.

THE INTERNAL RHYTHMS OF HIBERNATORS

by

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I. INTRODUCTION

It is important to appreciate that "hibernation" in mammals does not exist as a separate field independent of the rest of physiology and biology. When one asks the question, what are the mechanisms involved when a mammal enters or leaves the hibernating state, one has immediately entered into the fields of thermoregulation, the central nervous system control of homeostasis and behaviour and control systems operating within single cells. Furthermore, it is hoped that as a result of this review, it will be appreciated that one needs to enter into the field of biological clocks in order to fully answer the above question.

It is becoming increasingly clearer that in many species there is a cyclical (rather than a sustained) response to persistent cold. The period of this response cycle is approximately one year. The cycle is probably already programmed within the organism at birth, merely waiting for certain environmental clues to set the phase and allow life to take place at a slower rate in harmony with the natural winter season. But for some essentially unknown reason, this state can not persist indefinitely and it appears that another internal oscillation, with a basic circadian period, is intermittently used to bring the organism back to its higher rate of life.

We will first examine the field of circadian clocks and then consider the analysis of time-series data before reviewing the evidence, from a study of hibernators, for the point of view exposed above.

II. CIRCADIAN CLOCKS IN MAMMALS AND BIRDS

Within the last six years, numerous books or monographs (Bünning, 1963; Cloudsley-Thompson, 1961; Harker, 1964; Reinberg and Ghata, 1964; Richter, 1965; Sollberger, 1965) and the proceedings of symposia (Cold Spring Harbor, 1960; Ross Conference, 1961; New York Academy, 1962; Feldafing, 1965) have appeared on the subject of biological clocks. The large majority of animal studies have been concerned with activity cycles with a period of about 24 hours. Rhythms of activity with such periods are now commonly called "circadian", a term introduced by Halberg (1959). Certain aspects of the findings of this expanding research group are thought to be relevant to the problems of timing of the hibernation season and intermittent arousals from hibernation and will therefore be reviewed in the following section.

The activity rhythm is usually measured, in mammals, by the rate of turning a running wheel and in birds by the rate of perch-hopping during many consecutive days of recording. In Figure 1, taken from Aschoff (1965), the activity of a chaffinch (Fringilla coelebs) is demonstrated under three different conditions of

illumination. From the sixth to the thirty-second day of the experiment, the chaffinch was exposed to constant light (LL) of 0.4 lumen intensity. It is quite apparent that the onset of perch-hopping was delayed (occurred later) each new day, so that the activity cycle had a period greater than 24 hours, under conditions of constant weak illumination.

However, in constant strong light (120 lumens, days 48 to 66), the onset of perch-hopping activity advanced each day so that the activity cycle had a period of less than 24 hours under these conditions. As soon as a 24 hour light-dark cycle was imposed on the bird (days 1-5 and 33-47), the activity cycle was entrained; it started and stopped at dawn and dusk respectively.

Some terminology and the circadian rule. Under constant conditions of temperature and light (or darkness, DD), the measured period of the activity cycle is considered to be the "free running" period. Animals with predominant activity during the light portion of a light-dark cycle are said to be diurnal or day-active and those with a predominant activity during the night are described as nocturnal or night-active. The external oscillation (usually light-dark cycles) which synchronizes the free running rhythm is termed the "Zeitgeber". During free running cycles, the time during which a diurnal species is predominantly active is called the "subjective day" and the portion of the record, when at rest, is called the "subjective night".

The "circadian rule" states that the free running period is shortened in diurnal animals and lengthened in nocturnal animals, with increasing intensities of light (Aschoff, 1960; Hoffmann, 1965). This rule is demonstrated in Figure 1 where the chaffinch's free running period dramatically decreases when light intensity is increased from 0.4 to 120 lumens.

Some mammals are exceptionally accurate in the expression of their free running period. Perhaps the best demonstration of this is the onset time of the running wheel activity in the nocturnal flying squirrel, Glaucomys volans. DeCoursey (1961) found, for this species, that the free running period (in DD) of 16 individuals had a standard deviation ranging between + 2 to + 15 minutes during 48 test periods, each ranging from 10 to 113 days duration. The frequency distribution of the free running period (average cycle length) for this population of 16 squirrels is illustrated in Figure 2 (DeCoursey 1960a). The distribution appears normal (Gaussian) with about 75% of the test periods accounted for in the block of periods from 23:40 to 23:59 hours. There can be little doubt from data of this sort that the free running period is an indication of a self-sustained (internal) oscillator in the animal independent of subtle geophysical factors (linked with the rotation of earth and orbit of the moon), but potentially entrainable by

some. This view has been expressed by many researchers (Pittendrigh and Bruce, 1957. Aschoff, 1960. Bünning, 1963. Harker, 1964; Enright, 1965a) but is by no means universally accepted (Brown, 1965).

The mechanisms by which a Zeitgeber entrains the innate free running period is not completely clear. There are however certain phenomenological observations that are considered relevant to any "final" model of entrainment. If, for example, the light portion of a Zeitgeber is imposed on a flying squirrel during its subjective night, a phase delay occurs in the onset times of subsequent activity, until the activity onset arrives in a time zone just after lights-out (Figure 3, cases 1 and 4, DeCoursey, 1960a). If, however the light portion of a Zeitgeber is imposed on a squirrel during its subjective day, little change is seen in the onset time of activity, from the free running situation, until the activity onset arrives in a time zone just after lights-out (Figure 3, cases 2 and 3, DeCoursey, 1960a).

These results already suggest that a rhythm of light sensitivity exists within the organism, for a light signal applied during the subjective night causes a phase delay, while a similar signal applied during the subjective day causes little change. By using single "light shocks" of ten minute duration, applied at 4 to 25 day intervals, DeCoursey (1960b) could obtain a light-sensitivity curve for individual squirrels. In Figure 4, taken from DeCoursey (1960b), the amount of advance or delay of the onset of activity, in minutes, is shown as a function of light-shocks applied at different times of the squirrel's activity cycle. The form of these light-sensitivity curves (also called "phase-response curves", Pittendrigh, 1965) is a characteristic of the species with variations of amplitude (delay or advance) common between individuals.

There is one aspect of obtaining a light sensitivity curve in higher animals that is somewhat disturbing. The reader will note that there was no effect of the light for a period of about 10 hours (squirrel A) and approximately 14 hours (squirrel B). These periods are presumably coincident with the inactivity portion of the squirrel's circadian cycle. The squirrel must be asleep, at times, during this phase of the activity cycle. Is the light signal not being severely attenuated during sleep, due to the closure of the eyelids and probable upward rotation of the eye? If the light signal is not sensed during sleep, one would not expect a response on the part of the animal to it. To the best of my knowledge, the control experiment of delivering a light shock during the inactive phase but with assurance that the squirrel is alert and thereby receiving the signal has not been described. Nevertheless one cannot use such arguments to explain the changing responsiveness to light shocks applied during the running phase of the activity cycle. These considerations are of some importance to

the analysis of periodicities in the arousal times from hibernation, as will be discussed in a later section.

Wever's model for circadian rhythms. A model is of some use in that it synthesizes from the body of data a few concepts or rules and restricts itself to an explanation of these in terms of well founded physico-chemical and mathematical principles. From a model can come predictions; the most useful model is the one whose predictions can be tested by an experiment.

In Fig. 5 taken from Wever (1964a, 1964b, 1965) three concepts are put together and it can be shown that a simple prediction emerges. The concepts are: There is a self sustained oscillation in the organism (of which a single sinusoidal cycle is shown under three different conditions in Figure 5). The oscillation has a "mean value", referenced to a stationary baseline, such that equal areas of the oscillation occur above and below the mean value. There is a threshold, which in Wever's model is fixed. When this threshold is exceeded by some portion of the oscillation (shaded area), activity on the part of the organism can be expressed. The activity time (portion of the oscillation above threshold) is abbreviated alpha (α) and the rest time (portion of the oscillation below threshold) is abbreviated rho (ρ). It can be seen from Wever's figure that as the mean value of the oscillation increases, relative to threshold, the ratio $\alpha:\rho$ increases as well as A, the total amount of activity (the area of the oscillation above threshold).

The prediction that emerges is that if light were to increase the mean value of the free running oscillation then both the ratio $\alpha:\rho$ and the total amount of activity (A) should increase. In Figure 1, it can be seen that $\alpha:\rho$ increases with a shift in light intensity from 0.4 to 120 lumens and in Figure 6 that the total amount of perch-hopping activity of the chaffinch increases with a shift in light intensity from 1.8 to 120 lumens.

Wever's quantitative model derives from the well known Van der Pol equation which he has modified into the form (Wever '65) for circadian periods:

$$\ddot{y} + 0.5 (y^2 + y^{-2} - 3) \dot{y} + y + 0.6 y^2 = \ddot{x} + \dot{x} + x \quad (1)$$

in which y is the variable, oscillating as a function of time, and x is the controlling variable (light intensity) which may be a function of time (when a Zeitgeber). The first and second derivatives, with respect to time, are shown as (\dot{y}, \dot{x}) and (\ddot{y}, \ddot{x}) respectively.

When this equation is solved for different values of a time-independent x , the system can be seen to oscillate within a restricted range of x (0.847 to 3.189) as in Figure 7. The most interesting aspect however is that the steady state circadian period shortens as x increases. If the sign of x is made positive for diurnal organisms and negative for nocturnal organisms, then

Wever's modification of the Van der Pol equation can be said to predict the circadian rule mentioned earlier: Increasing light intensity shortens the circadian period in diurnal and lengthens the period in nocturnal organisms.

III. THE ANALYSIS OF STATIONARY TIME SERIES

Three types of analysis of data consisting of stationary time series (non-damped) will be reviewed here: a) Periodograms, b) Autocorrelograms, c) Power Spectra. With each of these analytic techniques, dominant frequencies can be extracted but the statistical properties of such frequencies remain to be completely resolved.

a) Periodograms. Imagine a stretch of data with a biological variable (such as body temperature) studied as a function of time. Let us suppose that the length of data covers a 28 day period. If one suspected a 24 hour periodicity in the data, one would divide the 28 days of data into 28 equal periods (trial period) and essentially add the 28 curves after arranging the curves synchronously one under the other. Each point in the resultant single curve would represent the sum of 28 data points collected, the trial period apart, (24 hours -- and hence at the same time of solar day). On dividing each summed data point by 28, one obtains the mean value for that sample period. This mean curve would have a range (minimum to maximum mean value) and a standard deviation (σ):

$$\sigma = \sqrt{\frac{\sum_{i=1}^N (Y_i - \bar{Y})^2}{N}} \quad (2)$$

where Y_i = mean value of data points synchronous for one trial period,

\bar{Y} = mean value of all mean data points $Y_1 \dots Y_N$

N = number of data points in trial period (i.e. sampling frequency x time duration of trial period).

If one repeats this operation on the 28 day length of record but now dividing the record into 25 hour trial periods one obtains a new mean curve with perhaps a new range and standard deviation. A periodogram is some measure of the strength of a function at variously scanned trial periods.

The standard deviation is a more useful measure of the "strength" of a periodic function than the range. Hence one normally obtains a periodogram by making a plot of the standard deviation as a function of the scanned trial periods. Koehler et al., (1956) were among the first to apply periodogram analysis to biological variables studied during long time periods. Enright (1965b) shows the type

of calculation that is performed to obtain a periodogram and in re-analyzing data published, primarily from Brown's laboratory (1965a), concludes that the vast majority of the new analyses indicates a circadian period rather than a solar day or lunar day period.

In Figure 8, a periodogram is demonstrated for the brain temperature of a golden-mantled squirrel, Citellus lateralis while in a cold environment. The length of data, on which the periodogram was based was 28 days (March 1-28). Brain temperature was measured every 50 seconds, from an implanted thermocouple (Strumwasser et al., 1964) by an automatic data acquisition system. Recordings were made on magnetic tape in digital format, from which IBM cards were generated prior to analysis.

The periodogram clearly indicates a major peak near 24 hours which is to be expected since the squirrel was in the non-hibernating state throughout but with an applied Zeitgeber (LD 12:12).

b) Autocorrelograms. Consider the same stretch of original data, discussed above, the total length of data being 28 days (T). Two identical plots of this data are arranged one under the other, synchronized in time. The ordinates at each synchronous time point are multiplied, then the resultant new curve is summed along the time axis and the mean computed by dividing the grand sum by the number of data points, m ($m = \text{sampling frequency} \times T$). Thus from this first operation, a single number is obtained with units (variable)² at "lag" zero. This number is the autocorrelation function at lag zero. This same operation is repeated with one of the two identical plots moved along the time axis a discrete interval (lag 1), usually T/m . Again this operation is repeated after moving the curve forward through the same interval (lag 2) and so on until the two curves are no more than $T/2$ time units out of phase.

Reflection on this series of operations will show that when the two curves are displaced in time by exactly one period of the oscillating variable, even if it is buried in some noise, the autocorrelation function will bring out the cycle and peak. If the record contains only random "noise" there should be no lag at which the autocorrelation function increases to a peak, for all frequencies, within a band limit, will be more or less equally represented throughout the extent of the record. The autocorrelation curve is always at a maximum at lag zero, since in this position all products are really the square of the original function giving rise to only positive numbers, whose mean value must then be larger than the autocorrelation function at any other lag.

In the notation of calculus, the autocorrelation function A (d) of a time function $y(t)$ is given by:

$$A(D) = \frac{1}{T} \int_0^T y(t) \cdot y(t + D) dt \quad (3)$$

where D is the lag or delay interval.

The algebraic operations can be summarized in the following formula:

$$A(d) = \frac{1}{m-d} \left[\sum_{i=1}^{m-d} y_i \cdot y_{i+d} \right] \quad (4)$$

where m is the total number of data points, obtained at equal time intervals, and d is the lag interval number incrementing by one for T/m time units and i is the index number of the data point.

In Fig. 9, the autocorrelation function is demonstrated for a 28 day record (March 1-28) of brain temperature of the same golden-mantled squirrel mentioned under periodogram analysis. The mean brain temperature obtained over the 28 day period was subtracted from the original data points prior to the autocorrelation. As is evident from inspection of the record a strong circadian component is present.

To obtain the mean circadian period from the autocorrelogram, the lag, in days, at each cycle peak has been graphed as a function of the cycle number (Figure 10). A least squares regression was computed for the line shown through the data points. The slope of this line gave a circadian period of 23.92 ± 0.10 hours, the range indicated being for a 99% confidence limit.

c) Power Spectra. The power spectrum is a Fourier transform of the autocorrelogram (Blackman and Tukey, 1958). It represents a quantification of the various frequencies already exposed by the autocorrelogram. The advantage of a power spectrum compared to the autocorrelogram is that it quantitatively indicates the relative strengths of the various frequencies present in the original data. Power spectral analysis was first applied to the biological problems of circadian systems by Halberg and Panofsky (1961 a,b).

The product of cosine waves of different exploring frequencies (over the range of interest) and the autocorrelogram are first obtained. The mean value of this product, over the whole autocorrelogram, as a function of the exploring frequency is the power spectrum. Halberg and Panofsky have used the term variance spectrum instead of power spectrum because the ordinate represents variance per unit frequency. Excluding the spectral estimates (S) at lag 0 and 1, S can be computed from

$$S(r) = \Delta \tau \left[A(0) + 2 \sum_{d=1}^{l-1} A(d) \cos \frac{r d \tau}{l} + A(l) \cos \pi r \right] \quad (5)$$

where r = integer indicating the multiple of the frequency of the exploring cosine wave,
 Δt = lag increment in time units,
 and l = the last of the autocorrelation lags, usually not exceeding the time interval 0.1T.

In Figure 11, the power spectrum for the data of Figure 9 is illustrated. It is quite clear now that the predominant frequency is the circadian one; however shorter periods are evident around 8 and 12 hours, perhaps representing the duration of sleep or/and activity.

Comparison between the various analytic techniques. In Table 1, a comparison is made of the three techniques described above (periodogram, autocorrelation and power spectral analysis) applied to twenty weeks of continuously recorded brain temperature from a golden-mantled squirrel, Citellus lateralis. This long stretch of data was divided into five portions, each 4 weeks long. While the circadian period is evident with all three techniques, small differences in the estimated value are apparent between the three techniques (Table I). In general the power spectrum gives the highest values of circadian period while autocorrelation appears to give the lowest.

Which technique is the most reliable? That question can not be answered at the present time. The answer can only come from a study of a model system, that is, one in which the experimenter is in control of all the variables. For example, sinusoidal waves of a fixed frequency (artificial signal) can be generated and "noise" added to it. The ratio of signal:noise can be systematically varied and the three analytic techniques applied. One can then tell which technique comes closest in extracting the known signal frequency over the widest range of signal:noise ratio. All of this may sound very straight forward but there are problems due to the fact that noise can have many different qualities besides an amplitude or more appropriately root-mean-square value. It is not clear, in my mind at least, what would be a good simulation of biological noise when dealing with circadian type periodicities.

IV. DURING HIBERNATION

It is now well known that under constant conditions of cold, with or without a light cycle and even in deafened animals (Strumwasser 1959a) that all hibernating mammalian species so far studied intermittently arouse (Lyman and Chatfield, 1955; Folk, 1957, 1960; Strumwasser, 1959a; Pengelley and Fisher, 1961; Pohl, 1961; Menaker 1961, 1964; Tucker, 1962; Fisher, 1964; Strumwasser et al, 1964). Evidence has been presented that at the start of the hibernating season, at least, the hibernation test drops in Citellus beecheyi are clock initiated for these arousals occur in

the same time zone as arousals from normal sleep, the majority of which anticipate the light-on signal. (Strumwasser, 1959a).

The problem as to whether all arousals from hibernation are initiated by a circadian clock or not is far from straight forward. We have already seen that circadian systems can be described in terms of the interaction between a sinusoidal-type oscillation and a threshold. During sustained deep hibernation, the amplitude of the circadian oscillation most probably decreases due to the depressing effect of the low core temperature. Perhaps it stays depressed or possibly begins to recover. Obviously if it stays depressed (below threshold) during deep hibernation, and if the threshold remains stationary, arousals due to the circadian oscillations could never occur! But what if the threshold progressively decreases during sustained hibernation due to the accumulation of metabolites or the decline of certain intracellular substrates or enzymes, then one would have a complicated interaction indeed and the time of arousal could not be predicted from just knowing the frequency of the oscillator.

It must then be clear that the mere fact that arousals from deep hibernation do not occur at the same solar time can not be used as evidence that the circadian oscillation is not involved in arousals from hibernation. Yet in 1965, Twente and Twente state for Citellus lateralis "The timing of arousal and re-entry seems to be random as no circadian patterns were discernible when individual records were analyzed."

Twente and Twente (1965) studied 31 squirrels between November 12 and March 15. These squirrels had thermocouples implanted subcutaneously near the lower rib and attachment to the recording apparatus was apparently made during the first hibernation. Continuous recordings were made from individuals until the animals disconnected themselves from the recording apparatus. A total of 158 arousals are reported in their Table I. This means that each animal had 5 hibernation cycles, on the average. As we will see later on, this number of hibernation cycles is less than the normal number present during just the test drop phase of the hibernating season (Fig. 16) for this species and only about 13% of the normal number of hibernations present in one hibernating season.

In Figure 12, we have made a histogram from the data in Table I of Twente and Twente (1965). The number of arousals occurring in 4 hour blocks starting from midnight is illustrated. The distribution is fairly rectangular as would be expected if:

- a) The circadian oscillation was not exactly 24 hours.
- b) There was a random distribution of circadian frequencies among the 31 different squirrels studied.
- c) There was no Zeitgeber (the conditions described were constant darkness).

In Figure 13, a similar histogram of arousal frequency as a function of clock time is illustrated based on continuous brain temperature recording from a single golden-mantled squirrel during its second complete hibernating season (Strumwasser, unpublished). The distribution of these 53 arousals from hibernation is quite rectangular. Since these arousals were recorded from a single individual and since there was a Zeitgeber (LD 12:12), one can only assume that the Zeitgeber was not influential and hence the circadian oscillation, if present, was free running and drifted across the solar day several times during one hibernating season.

A similar analysis of an individual thirteen-lined ground squirrel during its second complete hibernating season is illustrated in Figure 14. A total of 43 hibernations occurred during the 1962-63 hibernating season. If arousals were random with respect to solar time, then around 7 arousals should be found in each four hour block. Between 18:00 and midnight there was only one arousal during the entire hibernating season (out of an expected 11 arousals). About 42% of the total number of arousals (18 out of 43) occurred in the time block 08:00 to mid-day, this number represents 2.6 times more arousals than would be expected on the basis of a random distribution (7 out of 43).

On the basis of these results, it is reasonable to conclude that the circadian oscillation during hibernation of the thirteen-lined squirrel had a period close to 24 hours and that the animal was probably influenced by the Zeitgeber (LD 12:12). One can only say "probably influenced by the Zeitgeber" because apparent synchronization could occur with "dawn", during the hibernating season, if the free running period was very close to 24 hours. If the animal is really being influenced by the Zeitgeber then a shift in the Zeitgeber should influence the distribution. These experiments remain to be done.

From the results so far presented, we can conclude that a circadian oscillation persists during deep hibernation in the thirteen-lined squirrel and appears to be one of the factors responsible for intermittent arousals from this state. On the other hand, from the discussion, it should be apparent that negative evidence from a simple arousal frequency-distribution curve, of even a single individual, can not be used as conclusive evidence that the circadian oscillation does not persist during hibernation, or is not a factor in initiating arousals. In the presence of a Zeitgeber, negative evidence from the arousal frequency distribution can be used in concluding that the organism is uninfluenced by the Zeitgeber.

According to Twente and Twente (1964, 1965a,b) the duration of hibernation is proportional to the core temperature achieved during hibernation. Their evidence is based on the relationship between duration of hibernation and subcutaneous temperature, as

shown in Fig. 15, in the form of an Arrhenius type plot - where log of velocity of some process (in this case, arousal frequency) is shown in relationship to the 'independent' variable, reciprocal of the absolute (subcutaneous) temperature. The slope of this line multiplied by a conversion factor (4.6) gives the Arrhenius constant (μ) for the process under study in kilocalories/mole. No one can argue that the line in Fig. 15 is a close fit to the Twente's 12 data points, each point being an average taken from several squirrels, each having hibernated several times. From this Twente and Twente (1956b) conclude "It is tempting to assume, because of the linearity of the data plotted....., that the duration of a hibernating period is limited by a single regulating process which is operating similarly at all temperatures between 2° and 25°. Because the periods at the slightly depressed core temperature of 36°C fit extrapolations of the lines of both figures, it is tempting to suggest that a relationship between hibernation and sleep may exist."

Because of the importance of the Twente's conclusions, further facts from our own experiments will be detailed here. Strumwasser et al. (1964) reported on a study of individual squirrels in which brain temperature was continuously recorded for periods over one year. The techniques can be found in the original report and will not be detailed here. In Table 2, the results of extending this study to 3 seasons are detailed.

Two individuals, one thirteen-lined and one golden-mantled squirrel, entered and aroused from hibernation a total of 123 and 113 times respectively during three hibernating seasons (1961-1964). Each animal averaged about 40 hibernation cycles each season. In Fig. 16 there is graphed the relationship between hibernation duration and brain temperature for the golden-mantled squirrel during the second season (September 11, 1962 to May 6, 1963). The relationship shown is typical for both individuals of the two different species during each of the three seasons.

Consecutive hibernations tend to form groups, each having a different (hibernation) duration-temperature relationship. Of the 53 hibernations during the second season, only the first twelve hibernations, about 23% (events 23-34) showed a duration dependent on the level of brain temperature achieved. These first twelve hibernations are clearly "test drops" (Strumwasser 1957, 1959a) since brain temperature was successively lowered each hibernation cycle. Group 2 (events 35-47) consisted of 13 hibernations in a reasonably tight group but far below a line extrapolated through the first group. Group 3 (events 48-67) consisted of 20 hibernations with a large vertical range. This group had a wide range of hibernation durations ($\sigma = 1.29$ days), despite a narrow band of achieved brain temperatures ($\sigma = 0.2^\circ\text{C}$), as can be seen from Fig. 16

and Table 3. Finally group 4 (events 68-75) consisted of the last eight hibernations of the season during which time the lowest brain temperatures were achieved followed by a return to the non-hibernating state via the range of test-drop temperatures but in the reverse direction.

On the basis of studying individuals over entire hibernating seasons, we cannot agree with the statement of Twente and Twente (1965a) that "the duration of hibernating periods was relatively constant and predictable for individuals at given (core) temperatures." Their statement is based on a clearly short hibernating season (163 hibernations among 31 squirrels) during which time the animals were exposed to presumably different environmental temperatures. In Table 4, taken from Twente and Twente (1965), it can be seen that eight squirrels were placed in cold room B on November 13 and core temperature is reported as 6°C (average?) for the 36 day period. In the next 21 day period (December 18-January 7), a core temperature of 10°C was reported for 7 squirrels (presumably the same original population); the environmental temperature must have been changed! In the last period reported for cold room B (January 8-February 7) a core temperature of 4°C is reported for eleven squirrels during the 31 day period. Hence the first population was first exposed to a low temperature for about 36 days, then a higher temperature for 21 days. Finally a new population (perhaps also a part of the old population) was exposed to the lowest temperature in the last 31 day period. Since each individual squirrel hibernated only about 8 times during the total cold room B period reported $(\frac{19}{7} + \frac{23}{11} + \frac{23}{8})$, all of the data of Twente and Twente (1965) could

have dealt with a test drop period at each new environmental temperature. The test drop period, as we have seen earlier, occupies less than 25% of the total number of hibernations during a normal hibernating season. The generalization of the Twentes' that a "single (temperature-dependent) regulating process" may be operating to control the duration of hibernation seems quite unrealistic and later we shall propose three factors - a temperature dependent threshold, a circadian oscillation and a seasonal modulator - as possibly accounting for the duration of individual hibernations.

A further analysis of the duration/temperature relationship was performed to test for possible non-linear relationships. The points in Fig. 16 have a superficial appearance of falling on a hyperbola. A hyperbolic relationship is well known among several pairs of biological variables (e.g. the current strength-latency relationship of a single nerve fiber). In a hyperbolic relationship the product of the two variables is a constant; e.g. in the strength-latency relationship, current x latency (time) is a constant value,

with units of electrical charge (coulombs). However, the product of hibernation duration and brain temperature is not a constant (Fig. 17 and Table 3) the means for the four groups varying between 7.27 and 26.7 days °C.

Three peaks are evident from the frequency distribution of the products, at least 75% of each peak being composed of a pure group (in order of an increasing value of the product-Groups 1, 3 and 2). It is possible that the 3 peaks of the duration-temperature product are reflections of three zones each with an overlapping gaussian distribution, but more data is needed before any conclusions can be drawn. As is evident from Table 3, although the mean temperature of groups 2 and 3, respectively, are similar (5.0 ± 0.4 °C, 4.8 ± 0.2 °C), the duration of hibernations are not (5.38 ± 0.96 , 3.61 ± 1.29 days).

Using only the first group (test drop stage) of the second and third hibernating season of a single golden-mantled squirrel, an "activation energy" of 27,900 calories/mole is obtained for the second season and 27,500 calories/mole for the third season, but there is a sizeable scatter (Fig. 18).

A three-factor theory of arousal frequency. In Wever's model of a circadian system, which was reviewed earlier, the components consisted of a sinusoidal-like oscillation with a mean value dependent on light intensity and a threshold that is considered stable (see Fig. 5). The relationship between the mean level of the oscillation and threshold determined the activity time (α) and amplitude (portion of oscillation above threshold) and rest time $-\rho$ (portion of oscillation below threshold). It seems reasonable to propose that the circadian oscillation continues during hibernation but that it is below threshold due to the depressive effect, much of the time, of cold temperatures on the mean level. An arousal occurs when the oscillation crosses threshold. For this to happen, it is assumed that the threshold decreases as a function of time and the temperature level achieved in hibernation (the lower the temperature, the slower the rate of decrease of threshold). With each arousal, the threshold gets "reset" to its original high level. The decrease of threshold could be due to one or a combination of many factors such as an accumulation of metabolites and a decrease of required intracellular templates (Strumwasser 1959b, Strumwasser et al., 1964) perhaps messenger RNA (Brenner et al., 1961). Lactic acid, urea and an altered blood pH or pCO_2 appear to be ruled out (Fisher, 1964; Twente and Twente, 1964 Lyman and Hastings, 1951). Experiments testing the hypothesis of a decrease of required intracellular templates, as a stimulus for arousal, are lacking.

During the aroused state (in between hibernations), the threshold is rapidly reset to its normal high level by a more

efficient homeostasis, operating at a higher metabolic rate. Figure 19 summarizes the model showing the depression of mean level of the oscillation with entrance into hibernation and the sawtooth-like decrease of threshold during the course of hibernation. Arousal is initiated when the threshold crosses the oscillation. During the aroused state, the threshold is rapidly reset to its high level.

The limitations of a two factor model are obvious: while it can explain long durations of hibernations by "skipped days" (circadian oscillation below threshold), it demands that the number of skipped days be dependent on the slope of the threshold decrease and at any fixed core (brain) temperature this should be constant. Hence the variable duration at a fixed core temperature still requires some other explanation. A third factor is needed in this model and that is a "seasonal" modulator. Since this will be discussed in more detail in the final section, only the pertinent portion of the argument will be presented here.

The seasonal modulator accounts for the fact that hibernation durations are not a constant function of the brain temperature achieved. During the second quarter of the hibernating season, the duration of hibernation is longer than during the last quarter, at equivalent brain temperatures (Figure 16). This and the fact that the ratio, duration of hibernation/duration of arousal, rises to a peak and then declines during the hibernating season (Strumwasser et al., 1964; Figure 25) suggests the following: The circadian oscillation is changing its amplitude/temperature relationship during the hibernating season, becoming less sensitive to temperature as the season progresses. In addition, or alternatively, the threshold may progressively become more sensitive to temperature during the season, declining at a faster rate as a function of time, at the same temperature, later in the season. Either or both of these factors would shorten the length of hibernation toward the end of the season, by allowing the threshold to cross the circadian oscillation sooner. As a matter of fact both of these factors could help bring the hibernating season to a close.

Circadian and ultra-dian oscillations in the brain and single neurons. The demonstration of a circadian or other macro-period oscillation in the brain would certainly help strengthen the argument that it plays a role in arousal from hibernation. In Figure 20, the autocorrelogram of 50 days of brain electrical activity, recorded from the septum of *Citellus beecheyi*, is illustrated. The electrical activity from this brain region was rectified and integrated, a number proportional to the integral was generated and recorded each hour during the 50 day period. As is clear from the autocorrelogram of this time series, a circadian oscillation exists with a mean period of 24.70 ± 0.12 hours (Figure 21).

In the amygdala of Citellus beecheyi, an oscillation in electrical output with a 5 to 6 hour period can be recorded during deep hibernation (Strumwasser et al., 1963). Arousal from hibernation always occurs when the amplitude of this oscillation becomes critically large (Figure 22). As is also obvious, the amplitude of the amygdaloid oscillation progressively grows during deep hibernation, preceding by about 20 minutes small oscillations in the recorded brain temperature.

These oscillations with macro-periods have now been demonstrated at the level of a single neuron in an invertebrate. One of the identifiable neurons in the isolated parieto-visceral ganglion of the sea hare, Aplysia californica, emits a burst of action potentials every few minutes. The spike output rate of the cell is, however, a function of the time of day and the previous history of the organisms exposure to light-dark cycles (Strumwasser, 1963, 1965 a,b). Figure 23 (Strumwasser 1965 a) shows the "activity" of the cell (spike output rate) as a function of clock time. The intracellularly recorded spike output rate very clearly peaks around the projected transition of dark to light on the first day and somewhat later on the second day of recording. Evidence, presented elsewhere (Strumwasser, 1965 a), indicates that both the microcycle (bursting) and the macrocycle (circadian period) are controlled from within this single neuron and are hence endogenous events, subject however to modulation by environment signals.

The role of a circa-annual clock. "Quite evidently we know comparatively little as yet about the mechanisms governing the onset and ending of hibernation". This quotation comes from Pengelley and Fisher (1963) who have made the most significant observations concerning the onset and termination of the hibernating season in any of the hibernating species studied. Pengelley and Fisher observed a colony of individually housed golden-mantled squirrels (C. lateralis) daily, during a two year period, for signs of hibernation. Each week the total food consumption and the weight of each animal was recorded. Figure 24, (Pengelley and Fisher, 1963) demonstrates that under conditions of constant cold (32°F) and a fixed light cycle (LD, 12:12), the squirrel did not continue hibernation but cycled back into the state twice, at about one year intervals. It is also interesting to note that the body weight reaches about the same peak value just prior to the onset of the three hibernating seasons, due in part to an increased food consumption. We have confirmed Pengelley and Fisher's annual clock in 3 species of Citellids - lateralis, tridecemlineatus and beecheyi (Strumwasser et al., 1964) - by continuous recording of brain temperature from individual squirrels over several years.

In Figure 25, the ratio of hibernation duration/arousal duration for two successive hibernating seasons in an individual of C. tridecemlineatus are shown as a function of calendar month. The

environment was maintained at 5°C and with a constant light cycle (LD, 12:12). Although the general shape of the ratio function is similar for the two consecutive seasons, the second season is clearly shifted to the left, and the third season is also phase advanced (see Table 3). Each hibernating season for the thirteen-lined squirrel lasts about seven months but the phase advance of the onset time produces a period less than one year (just nine months for the second to third season). These measurements are consistent with Pengelley and Fisher's observations that the annual cycle in *C. lateralis* is consistently less than one year whether the animal is maintained in a constant cold (32°F) or warm (70°F) environment. Pengelley and Fisher's demonstration that an annual cycle of hibernation, body weight and food consumption also occurred during a two year period, when the environment was maintained at 70°F, conclusively demonstrates the endogenous nature of this cyclical activity.

One thing leads to another. It would seem reasonable to consider the annual cycle of hibernation, under laboratory conditions, as consisting of a sequence of a series of events. The accomplishment of each end-point in a "reaction" triggers the next reaction which leads to another end-point which then triggers the next reaction and so on.

In this way the cycle probably consists of preparations for hibernation achievement of a certain critical metabolic state (one index being body weight) hibernation utilization of metabolic reserves termination of hibernation achievement of the reproductive state (reproductive behaviour) preparations for hibernation. Perhaps the short circa-annual period, observed in the laboratory, of the hibernation cycle, is due to the absence of the social interactions which would normally take place in the field at the end of the hibernating season, perhaps thereby extending the active reproductive state.

It is clear that much research remains to be done on the internal rhythms of hibernators particularly on those pertinent central nervous system mechanisms which at the cellular level span circadian and perhaps circa-annual periods.

Acknowledgements. During the period mid-1960 to mid-1964 the research of the senior author was supported in part by contract Number DA-49-193-MD-2119 from the U.S. Army Medical Research and Development Command. The author's hibernation research is being presently supported by a grant from NASA (NGR 05- 002- 031). The authors wish to acknowledge help with data analysis from Mr. Octavio Perez and Mr. Calvin O. Henson and with mechanical instrumentation from Mr. James J. Gilliam.

A FORTRAN IV COMPUTER PROGRAM TO CALCULATE POWER SPECTRA

by

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SUMMARY

This program accepts an array of previously calculated autocorrelation coefficients and obtains both raw and smoothed power spectral density estimates. It also calculates the equivalent chi-square degrees of freedom. The method used is that of Blackman and Tukey for equally-spaced discrete data. Smoothing is done by "hanning" the raw estimates.

INPUT

The first input parameter is the number of problems. Input for each problem includes a descriptive problem label, the size of the array of autocorrelation coefficients, the duration of the test measured in lag increments, the duration of a lag increment measured in time units, the name of the time unit, the input format of the autocorrelation coefficients, the problem type number, and in one case the starting value of the frequency variable. These parameters are followed by an array of autocorrelation coefficients.

The problem type is either 1, 2, or 3. For type 1 problems the analysis is performed exactly as in Blackman and Tukey. The number of spectral estimates obtained is then the same as the number of autocorrelation coefficients used as input. For type 2 problems a type 1 analysis is first performed. Then three interpolations are performed in the region of the peak to locate the peak more accurately. Each interpolation consists of calculating 21 new estimates in the neighborhood of the known peak. The frequency step is reduced by a factor of 10 with each iteration. For type 3 problems the standard analysis is omitted and interpolations are begun at a frequency which must be specified. The main purpose for including a type 3 option was to provide a means for locating the secondary peaks. Sometimes the calculated peak is at zero frequency. In this case the peak of interest may be located by specifying a type 3 problem.

EXPERIENCE WITH THE PROGRAM.

The power spectra program was checked out by comparing its results with results of a hand calculation on a type 1 sample problem. The iterated cycles are self checking because some points in each new cycle duplicate points in the previous cycle.

The computer at Cal Tech is an IBM 7090-7094. Execution time for a type 2 problem with 167 lags is 17 seconds. The time varies roughly as the square of the number of lags. As the program is currently set up it will accept up to 500 lag coefficients.

A number of production runs were made in order to assess the effects of two parameters on the calculated dominant period of the power spectra. These parameters are the averaging interval for data entering the autocorrelation program and the maximum lag in the autocorrelation coefficients used in the power spectra.

The effect of varying the length of the data samples was studied using 26 days of temperature data having a dominant period near 24 hours. Separate autocorrelations were performed using data averaged over 1/6, 1, 2, and 4 hour intervals with a lag increment equal to the averaging interval. The power spectra was calculated in each case using lags up to about one tenth the test duration. The results are given in table 1.

TABLE 1

| Averaging interval for data to be autocorrelated, hours | Period at peak in power spectra, hours |
|--|---|
| 0.167 | 24.23 |
| 1.0 | 24.24 |
| 2.0 | 24.29 |
| 4.0 | 24.78 |

Very little change in the calculated dominant frequency is obtained when the data sample ranges between 1/24th cycle and one-sixth of that.

The effect of varying the size of the maximum lag was investigated using 28 days of temperature data having a spectral peak in the neighborhood of 24 hours. The autocorrelation was calculated using half-hour data averages and half-hour lag steps. Results are given in table 2.

TABLE 2

| Maximum lag, days | period at peak in power spectra |
|----------------------|---------------------------------------|
| 1 | 23.09 |
| 2 | 23.66 |
| 4 | 23.93 |
| 7 | 23.95 |

In this case a maximum lag of at least four times the period is required to produce a power spectra peak which is relatively unchanged if the analysis is repeated with more lags. If the maximum lag is varied in steps smaller than whole cycles, the variation of the calculated period is not monotonic with increasing maximum lag.

A PROGRAM TO COMPRESS, REFORMAT, AND
SUMMARIZE THE MAGNETIC TAPE RECORD OF
SEVERAL INTERMIXED TIME SERIES

by

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I. GENERAL PURPOSE

The objective of this computer program is to provide a compact and convenient means for storing large quantities of sequential digital information, principally time series. The data will be analyzed for periods ranging from minutes to years.

For this particular installation, up to nineteen different experiments (input channels) can be monitored. A digital tape recorder is used to record the times, channel numbers, and data values as each channel is scanned, sequentially, at intervals ranging from 5 seconds to 5 minutes. The recording medium is magnetic tape. The information is digital and is recorded in BCD form (low density) compatible with an IBM-7094. The recorder, however, is in no way linked to the computer.

The program uses this tape as input, producing a new tape in a more suitable format as output. It checks the data for obvious errors and sorts the entries by channel number, grouping them into hourly aggregates. A new storage tape is produced which contains all the valid original data as well as hourly summaries and identifying information. Six or seven original source tapes can be compressed into one storage tape since most of the record gaps are eliminated and the recording is done at a higher bit density (556 cpi).

For more economical analysis when only a coarse degree of resolution is required, the program produces a deck of punched cards containing 5-minute averages for each channel which is being scanned.

Monitoring at 10 second intervals has been in progress 24 hours a day for the last 8 months, with only occasional minor interruptions for changing tapes, maintenance, or repair. Non-systematic recording errors have been extremely rare, but systematic errors requiring programing modifications for their accommodation have never been totally eliminated.

The author has had some experience processing data from similar but less ambitious projects recorded on punched paper tape. In his opinion, this system is far superior, particularly with regard to reliability, storage capacity, convenience, and most important of all, a minimum of repair and maintenance.

II. INPUT TAPE FORMAT

The magnetic tape transport is the Digidata model DSR 1420. For greater reliability the recording is done at low density (200 characters per inch) on tape rated at 556 characters per inch.

An entry consists of two 6-digit words. The first word gives the time of the entry in hours, minutes and seconds. The two high-order digits of the second word give the channel number. The remaining four give the data value.

During normal operation, an arbitrary selection of the input channels from numbers 1 thru 12 is scanned sequentially at the rate of one input channel every 10 seconds. Since these input channels may be individually selected or deselected, the scanning rate for a

particular channel will vary inversely with the number of channels selected. The user also has the option of increasing the scanning rate to one reading every 5 seconds.

Once every 5 minutes, input channels 13 thru 19 are scanned as a block. They may not be individually selected. These seven channels serve as counters, and are automatically reset to zero after being scanned. A systematic error occurs if one of the first twelve channels is scanned while these seven are being scanned: one of these seven is not recorded on tape, nor is it reset to zero. Recording of the data from the interfering scan is delayed until channels 13-19 have been recorded.

A record gap is always written between successive scanning cycles of the first twelve channels. Thus the record length is variable, having a maximum of 38 words. Such short records mean a significant portion of the tape (up to 50%) contains no information. This places a severe limitation on tape capacity and increases the processing time. However, short records have the advantage that illegible records may be discarded without much loss of data. The incidence of illegible records has been negligible.

At present, we can record about 2 weeks of data (over one-quarter million words) on one $10\frac{1}{2}$ inch reel of tape. The end of the tape is indicated by three consecutive END-OF-FILE'S which the user writes on the tape before he removes the reel from the tape transport.

III. THE PROGRAM TO PROCESS THE INPUT TAPE

A. Initial positioning of the Input and Storage Tapes.

An indispensable aid in processing the tape is a printed strip-paper record containing every entry. It is used to identify the first and last entries in each tape and the beginning and ending entries for patches of erroneous data which must be skipped. (It is also necessary for the early detection of machine malfunctions and as an aid in debugging.)

To position the tapes and to check that the correct tapes have been mounted, the last day and hour of the storage tape and the first entry to be processed from the input tape are read into the computer from a punched card. The tapes are searched for a match before processing can begin.

The storage tape is read and written with FORTRAN IV binary READ and WRITE statements. The input tape is read by a MAP tape handling routine developed by the Biosystems department at the Caltech computer center. The tapes are positioned on different computer channels. Records are alternately read from each tape to take full advantage of the buffering ability of the computer.

B. Reading, Checking, and Binarizing Data from the Input Tape.

The data from the input tape is processed one record at a time. The hour of each entry must be less than 24, the minutes and seconds less than 60. Each time entry is converted from hours, minutes, and seconds in BCD form to the nearest ten-thousandth of an hour in integer

binary form. Each BCD character in the channel number and data value must be numeric. Data entries are converted to six-digit binary integers. The channel number must be less than 20. If any of the above criteria are not met, an error message is printed out and the entry is ignored.

Processing continues until three consecutive END-OF-FILE'S are read, or alternatively, the first two words of each record may be checked for a match with a two-word entry from punched cards identifying the terminal record. Thus processing may be stopped or started at any point on the tape.

C. Organizing a Storage Record

The program stores the data in hourly records. Because of omissions which must be filled from subsequent entries, the hourly processing is not initiated until the first six minutes of the next hour have been entered into the memory. The computer then sorts the data by channel number, preserving and checking the temporal sequence at the same time. A tolerance check is run on the first differences of selected channels. Omissions in channels 13-19 are filled by distributing the count from the next entry. The entries for each channel are counted and summed. Hourly averages are printed on the high speed printer. Five-minute averages are punched on cards, and finally, a new record is written on the storage tape.

Each storage record contains the following information: the year, the calendar day, the hour, the number of entries in each channel, the sum of the data values for each channel, and all the sorted binarized data values with their associated times correct to .0003 hr.

The tape is written in the binary mode at a density of 556 characters per inch. If the record to be written is the first hour of a new month, the program writes on a new storage tape.

Any sequential jump to a higher hour results in a record being written on tape for each hour that was skipped. These records indicate that no data was recorded during that hour. Any entry which has a smaller time than the previous entry is ignored unless the previous hour was 23.

D. Detecting Errors.

As a general rule, diagnostic messages are printed by the high speed printer as soon as errors are detected. Thus a record of the errors is interspersed between the printed hourly averages. Whenever possible, the erroneous data is ignored and processing continues. Limits have been placed on the number of error messages which can be printed per hour of data.

The following types of errors have been encountered:

1. A character is not a digit.
2. An impossible time value or channel number.
3. A jump to a smaller time value. This is usually an isolated entry consisting mostly of zeros. It seems to be the result of a false triggering of the recording device.

4. An omission in the scanning sequence for channels 13-19 (discussed above)
5. If channel 19 has been omitted and the next channel number is less than 10, a one is sometimes inserted in the ten's position of the channel number. As a precaution, whenever the entry following an omission of channel 19 has a channel number higher than 9, it is ignored.

E. Checking and Editing the Storage Tape (Not yet operative)

A storage tape can hold about 3 months' data. This means that about six input tapes can be compressed into one storage tape. Because of the cost involved in reading data near the end of the tape reel, we have been storing only one months' data per reel.

Two separate programs to check and edit the storage tapes are being written.

1. The Checking Program

As the tape is read, the calendar day and hour will be checked for sequential continuity. The data values for each channel will be summed and compared with the previously calculated sums from the tape record. Where possible, a tolerance check will be run on the data values and/or their first differences. The times associated with the data values must increase monotonically. If any of the above checks are not met, diagnostic messages are printed and the record is dumped. An option will be available for simultaneously copying the tape.

2. The Editing Program

Corrections, insertions, and deletions will be punched on cards. The computer will sort them into the order they will appear on tape. The tape will then be searched for the position at which the appropriate editing action is to take place. An edited copy of the tape will be produced which is then rechecked, and duplicated on a three-month storage tape.

IV. COMMENTS ON PROCESSING TIME

If no processing takes place, a reel of input tape (two weeks of data) can be read by the computer in four minutes. An input reel can be read and processed in six minutes. A month's data from a storage tape can be read in 1.5 minutes.

The entire program, with the exception of the routines to handle the input tape, was written in FORTRAN IV. It is realized that revising some of these routines and rewriting others in the MAP language would reduce the processing time, but the lower limit is apparently four minutes.

A more significant reduction in processing time could only occur if the reprogramming effort were combined with hardware modifications to increase the record length and the recording density of the input tape